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Abstract

Imprinting is a type of learning by which an animal restricts its social preferences to an object after exposure to that object. Filial imprinting occurs shortly after birth or hatching and sexual imprinting, around the onset of sexual maturity; both have sensitive periods. This review is concerned mainly with filial imprinting. Filial imprinting in the domestic chick is an effective experimental system for investigating mechanisms underlying learning and memory. Extensive evidence implicates a restricted part of the chick forebrain, the intermediate and medial mesopallium (IMM), as a memory store for visual imprinting. After imprinting to a visual stimulus, neuronal responsiveness in IMM is specifically biased towards the imprinting stimulus. Both this bias and the strength of imprinting measured behaviourally depend on uninterrupted sleep shortly after training.

When learning-related changes in IMM are lateralised they occur predominantly or completely on the left side. Ablation experiments indicate that the left IMM is responsible for long-term storage of information about the imprinting stimulus; the right side is also a store but additionally is necessary for extra storage outside IMM, in a region necessary for flexible use of information acquired through imprinting.

Auditory imprinting gives rise to biochemical, neuroanatomical and electrophysiological changes in the medio-rostral nidopallium/mesopallium, anterior to IMM. Auditory imprinting has not been shown to produce learning-related changes in IMM.

Imprinting may be facilitated by predispositions. Similar predispositions for faces and biological motion occur in domestic chicks and human infants.

This review will focus on filial imprinting, a phenomenon known since antiquity. It is characterised by an animal following and establishing a social attachment to an object early in life as a result of being exposed to that object. In natural circumstances that object is usually a parent. However, this is not necessarily the case and artificial objects can be powerful imprinting stimuli. Imprinting is most readily observed in precocial animals; that is, those which are relatively mature and mobile soon after birth or hatching. Accordingly, much research on imprinting has been conducted on domestic chicks and ducklings. Filial imprinting has, however, also been described in the blackbird, which is altricial¹, and in many other vertebrate species². Comprehensive reviews of the imprinting literature have been published²⁻⁷, as have accounts of work on the underlying neural mechanisms^{4,8-11}. I will concentrate mainly on imprinting research published after the last of these reviews, but will refer to earlier papers as the need arises. Unless otherwise stated, the term "imprinting" will refer to filial imprinting.

BOX 1 near here

An essential feature of imprinting is recognition: the identification of a stimulus that has previously been experienced (Mandler 1980; Brown and Aggleton 2001); see BOX 1. When an animal becomes imprinted to an object it learns characteristics of that object. The animal's social preferences, measured behaviourally, then give information about the learning that has occurred. In natural circumstances it is likely that the learning leading to a filial bond is complex. For example, there is the possibility of perceptual learning when the animal is not interacting with the imprinting stimulus and additionally of operant conditioning when it can. It is clear, however, that interaction with the stimulus is not necessary for the formation of a filial bond. Many laboratory studies have therefore simplified investigation by preventing such interaction.

There has been much enquiry as to what type of learning is involved in imprinting. Salzen and Sluckin¹² and Sluckin² proposed a form of perceptual learning whilst others¹³ have suggested that one component of the imprinting stimulus (such as movement) elicits approach as an unconditional stimulus and another component (such as colour) is a conditional stimulus that comes to elicit approach through classical conditioning (see ^{7,14,15} for critical discussion of these viewpoints). Bateson¹⁶ has argued that recognition memory is central to imprinting in the young animal and that

the same process may continue to function throughout the animal's life; such memory may represent a broad class of processes responsible for the formation of neural representations of the external world.

Connectionist models of imprinting drawing on behavioural and neurobiological data have been developed^{8, 17-19}, and implemented as artificial neural networks^{20, 21}.

THE SENSITIVE PERIOD FOR IMPRINTING

Imprinting is a characteristic of the young animal and is a means by which kin may be recognised in early life. The very survival of a hatchling may depend on recognising and keeping close to a parent, because social attachment to an unrelated animal, even a conspecific, may result in the young animal being attacked. Imprinting can be the means by which a sufficiently precise identification of a particular parent is made: Johnson and Horn²² have shown that domestic chicks, after several hours of exposure, acquire a preference for an individual adult hen over a novel individual. A neural correlate of this recognition process has been described²³. Later in life, when the animal attains its adult appearance, sexual imprinting is a means by which potential mates may be selected; litter/nest mates (which normally are closely related) may be recognised and avoided, as may animals that do not resemble those litter mates sufficiently to be likely productive breeding partners. In contrast, animals differing only slightly from litter mates are commonly sexually attractive²⁴. Recognition of familiar animals may thus be an important determinant of optimal outbreeding. There are times in the life of the animal when imprinting is particularly important and sensitive periods for both filial and sexual imprinting have been demonstrated^{25, 26}.

During the first few days after hatching, domestic chicks will explore a wide range of objects and typically narrow their social preferences, usually to a parent or siblings. As chicks develop social attachments, their attention is diverted from novel objects and eventually they become fearful of novelty. Imprinting – the acquisition of a preference for a familiar object – thus limits the acquisition of new preferences, not least because the consequent aversion to novelty restricts the chicks' experience of new objects. There is thus a sensitive period for filial imprinting that can be curtailed by the imprinting process itself²⁷. Domestic chicks deprived of visual experience at hatching will follow and become imprinted to visual stimuli if they are allowed sight of their environment within about three days of hatching. However, if they are kept in darkness a day or so longer, when now allowed visual experience they will vigorously avoid most objects if not everything they see, and strongly prefer a dark environment. This dramatic example of the sensitive period was noted by Spalding²⁸, who concluded that the sudden development of this strong aversion in chicks "could not have been the effect of experience; it must have resulted wholly from changes in their own organization". It is however possible that the chicks had, in effect, imprinted to darkness and avoided all visual stimuli simply because such stimuli were novel. See ²⁶ for a discussion of the nature and biological significance of behavioural sensitive periods.

The sensitive period for filial imprinting can be extended pharmacologically. Treating domestic chicks with the anaesthetic mixture ketamine/xylazine 10 h after hatching permitted the chicks, when exposed to a model of a hen ~ 8 days later, to acquire a preference for the hen over an alternative artificial stimulus (a red box); chicks exposed at the same time to the red box did not develop a

preference for either of the two stimuli but the results were interpreted as an extension of the sensitive period for imprinting rather than as a non-specific predisposition to approach the hen; this was because the preference for the hen was specific to the hen-trained group rather than occurring in all chicks, which would be the expected outcome for a predisposition^{29, 30}. Ketamine is an inhibitor of the N-methyl-D-aspartate (NMDA) class of glutamate neurotransmitter receptors, although a not particularly specific one. The more specific NMDA receptor inhibitor MK-801 had a very similar effect on the hen-trained chicks³⁰, more closely implicating NMDA receptors in maintenance of the sensitive period. Yamaguchi et al³¹ have shown that the length of the sensitive period for imprinting in domestic chicks can be extended to at least eight days by injection of the thyroid hormone 3,5,3'-triiodothyronine (T_3) on days 1-4 post-hatch. The efficacy of training with an imprinting stimulus can be enhanced by administration of T_3 , or impaired by inhibiting the production of T_3 from its precursor thyroxine. The study suggests that T_3 , the endogenous level of which rises around the time of hatching, initiates the sensitive period for imprinting and exerts a critical influence on the animal's capacity for memory formation during the sensitive period and thereafter.

It is not clear to what extent pharmacological modification of the sensitive period acts by affecting the relative attractiveness of familiar and novel objects, or acts on a developmental programme that is independent of experience. It would perhaps be surprising if such developmental programmes did not exist, and indeed the success of imprinting is correlated with developmental age^{32, 33}. Drawing on evidence from other systems, there are many candidate mechanisms for developmental control of a sensitive period^{34, 35} but in this particular instance knowledge is, as yet, incomplete.

NEURAL MECHANISMS OF VISUAL IMPRINTING

A considerable amount is known about the neural mechanisms underlying filial imprinting to a visual or auditory stimulus in the domestic chick. A restricted region within the forebrain has been found to be critical for imprinting on a visual stimulus. Originally known as the intermediate and medial part of the hyperstriatum ventrale (IMHV)⁸, it is now termed the intermediate and medial mesopallium (IMM)³⁶. Bilateral ablation of the IMM before exposure to an imprinting stimulus ('training') prevents imprinting³⁷. Bilateral ablation of this region ≤ 3 h after training renders chicks amnesic for the imprinting stimulus³⁸. The evidence for the special role of the IMM in imprinting, indicating that it is a site of memory for information about the imprinting stimulus, has been reviewed comprehensively by Horn⁸.

Biochemical and neuroanatomical studies

A number of biochemical changes indicative of synaptic modification in the IMM occur as a result of training with an imprinting stimulus. Imprinting training gives rise to an increase in the mean profile length of postsynaptic densities of axospinous (putatively excitatory) synapses in the IMM^{39, 40}. There is also an increase in the numerical density of NMDA receptors in the IMM that is specifically related to the strength of chicks' learning about the imprinting stimulus, inferred from behavioural measurements⁴¹. Although a change in a measure in the IMM following imprinting training is consistent with that change having a role in learning, the change does not by itself necessarily imply

such a role. A set of criteria have been developed that need to be satisfied before a given measurement (such as NMDA receptor binding) is accepted as probably having a role in learning⁴¹⁻⁴³. These criteria are based on a regression model⁴⁴ with the measurement in question (e.g. number of neuronal nuclei immunopositive for the activity marker Fos) as the response variable. A linear model is fitted, which includes: a measurement of the strength of learning; other variables that might influence the response (such as locomotor activity); and experimental factors such as training condition and brain region. One may then determine which components of the model are significant and thus associated with the response.

A common measure of strength of learning is the preference score, derived from a preference test conducted after a chick has been trained with an imprinting stimulus. In the preference test, the chick is exposed sequentially to the imprinting (training) stimulus and to an alternative stimulus which the chick has not previously seen. The preference score is then:

$$\frac{100 \times \text{approach to imprinting stimulus}}{(\text{approach to imprinting stimulus} + \text{approach to alternative stimulus})}$$

A chick that is strongly imprinted has a preference score near 100 and one that is not imprinted has a preference score near 50.

Figure 1 near here

The following steps are taken to interpret a putative learning-related response (cf Figure 1).

1. Determine whether the response and preference score are significantly associated. Partial correlation analysis can usefully supplement a regression model when it is necessary to correct for effects of covariates such as locomotor activity. Where an association between response and preference score is found, a linear fit is usually sufficient (see e.g.⁴³) but sometimes other simple functions are appropriate⁴⁵.
2. The regression model may be used to find the response corresponding to any chosen value of preference score (Figure 1). The response when preference score is 50 (no imprinting/learning) is compared with the mean value of untrained chicks. A finding of no significant difference implies that side-effects of the training procedure occurring independently of learning (vocalisation, arousal, locomotor activity, sensory stimulation, stress, etc) do not influence the response unless learning has occurred. If the response value predicted by preference score 50 differs significantly from the mean value of untrained chicks in either direction, the implication is that side-effects of training contribute to the response in the absence of learning.
3. The value of the response predicted when the preference score is the maximum preference score attained (indicative of strong learning), is compared with the mean value of untrained chicks (Figure 1). One may thus ask whether the response has changed sufficiently at the maximum measureable level of learning for the corresponding response to be significantly different from the untrained value.
4. An alternative to using preference score in the model is to substitute a factor in which trained chicks are separated into good learners and poor learners on the basis of preference score. One then compares the mean values of good and poor learners with

each other, and separately with the mean value of untrained chicks. In this approach, the strength of learning is estimated with a discrete rather than a continuous measure.

5. An association between the response and preference score is not necessarily the result of a causal relationship. It could simply be that chicks hatched with a higher level of the response measure are able to learn better, without the response being *affected* by learning at all. If this hypothesis were true (call it the 'predispositions' hypothesis), the sample variances of the trained and the untrained groups of chicks would each be estimates of the same population variance. Then if a significant association were found between the response and preference score, whether continuously measured or expressed in terms of good and poor learners, the residual variance after fitting the preference score term should be significantly *lower* than the variance of the untrained chicks. This is simply to say that a significant term in the regression model (a significant association between response and preference score) necessarily reduces the residual variance significantly. The predispositions hypothesis therefore makes a prediction which, by analysing residual variance in the regression model, permits this hypothesis to be distinguished from the alternative hypothesis, which is that the response is changed as a result of learning^{42, 43}.

Application of the above procedures has implicated a number of biochemical changes in the IMM as having a role in memory for the imprinting stimulus. Much of this information, including the time-courses of the changes, has been summarised by Horn¹¹

Figure 2 near here

Further information has since become available which, together with earlier results, is summarised in Figure 2. Also shown in Figure 2 is the timecourse of learning-related changes after training, which evidently progress from early rapid functional changes to more trophic, and thus presumably longer-lasting, changes approximately one day after training.

The protein kinase C (PKC) substrate myristoylated alanine-rich C kinase substrate (MARCKS) resides, in its unphosphorylated form, in the plasma membrane. There it is likely to bind and crosslink actin filaments, and sequester phosphatidylinositol-4,5-bisphosphate. Upon phosphorylation by PKC, MARCKS translocates to the cytosol, possibly causing reorganisation of actin filaments and adjusting the interaction between pathways controlled by PKC and calcium/calmodulin-dependent protein kinase II (CaMKII)⁴⁶. A further role of MARCKS is suggested by the observation that a peptide derived from the MARCKS effector domain inhibits nicotinic acetylcholine receptors in brain tissue⁴⁷. There is, in the IMM, a learning-related increase in MARCKS phosphorylation about 3.5 h after the start of training⁴⁸; about 1-5 h after the start of training, an increase in MARCKS mRNA expression occurs in good learners relative to poor learners⁴⁹. The amount of MARCKS protein in the IMM is increased in a learning-related manner about 24 h after training⁵⁰, at a time when levels of clathrin⁵¹, neuronal cell adhesion molecules (NCAMs)⁵² and amyloid precursor protein (APP)⁵⁰ have also been found to be increased in the IMM in a learning-related manner¹¹. Solomon et al⁵³ enquired whether the increase in amount of MARCKS protein at 24 h occurred in the unphosphorylated, membrane-bound form or the phosphorylated, cytosolic form. In the IMM only the amount of the unphosphorylated, membrane-bound fraction was associated with learning. No significant effects were observed in the posterior pole of the nidopallium (PPN), a control area employed in previous studies of biochemical

changes following imprinting¹¹, where no learning-related changes have been found. Solomon et al⁵³ suggested that the change in MARCKS at 24 h might stabilise relatively short-term modification of actin filament organisation resulting from the earlier change in MARCKS phosphorylation.

MARCKS was one of the proteins implicated by Solomon et al⁵⁰ using subtractive hybridisation to identify systematically the molecules in the IMM which, like clathrin and NCAMs, have been implicated in memory for the imprinting stimulus ~ 24 h after training. More than 50 partial cDNA clones were identified corresponding to genes that were up- or down-regulated in good learners rather than poor learners. Five further proteins were selected for detailed investigation to determine whether the amounts of these proteins changed in a learning-related manner. These proteins were: amyloid precursor protein (APP) (see above); subunits I (CO-I) and II (CO-II) of the mitochondrial enzyme cytochrome c oxidase; α -fodrin; and the chaperone protein HSP90. Amyloid precursor protein has been implicated in synaptic plasticity⁵⁴; the cytochrome c oxidase subunits were chosen because of the essential role of this enzyme in oxidative metabolism; α -fodrin was chosen because it had previously been implicated in synaptic plasticity^{55,56} and HSP90 because it is widely distributed in neurons⁵⁷ and may be involved in the recycling of synaptic vesicles⁵⁸. Training exerted differential effects on these proteins. There was a negative correlation between preference score and α -fodrin in the left IMM but no significant effect in the other regions studied (right IMM, left and right PPN). No significant experimental effects or correlations were observed for HSP90 in any region. However, the amount of CO-I changed with preference score in a learning-related manner in the left IMM⁵⁹ and amount of CO-II less strongly so in the same brain region. In addition to these effects, a strong correlation that was not dependent on training was found in the left IMM between levels of CO-I and CO-II. There was no such correlation in the three other brain regions studied (right IMM, left and right PPN).

In eukaryotic cells cytochrome c oxidase is assembled in the inner mitochondrial membrane. Subunits CO-I and CO-II, encoded in mitochondrial DNA, occur in equal amounts in the fully assembled enzyme⁶⁰. The amounts of the two subunits might therefore be expected to be correlated in any one brain region and this was found to be so in the left IMM⁵⁹. However, such close correspondence was not the case in the other three brain regions studied. Because the genes encoding CO-I and CO-II are adjacent to each other in the genome and are transcribed together, a lack of association is unlikely to be due to dissociation of expression of the two genes. In contrast, assembly of subunits, cofactors and metal ions into the enzyme complex, and stabilization of the enzyme pre-complex, involve many factors. Solomon et al⁵⁹ have suggested that, at the time of training within the sensitive period for imprinting, enzyme assembly is particularly efficient in the left IMM but much less so in the other three regions. CO-I and CO-II subunits would then be efficiently incorporated into the enzyme complex in the left IMM, giving a greater ratio of combined to uncombined subunits than in other brain regions. In those other regions, a greater proportion of subunits would remain unincorporated for longer and be correspondingly more vulnerable to degradation. Variability in the rate of degradation of the CO-I and CO-II subunits could account for the lack of correlation between their levels in the right IMM and left and right PPN. Solomon et al⁵⁹ suggested that the molecular mechanisms necessary for the coordinated assembly of cytochrome c oxidase may be precociously developed in the left IMM compared with other brain regions, enabling it to function efficiently as a memory store shortly after hatching.

Assay by cDNA microarray has shown imprinting to be followed by the up- or down-regulation of many genes ~ 3 h after training. Expression of microtubule-associated protein 2 (MAP2) is particularly strongly up-regulated⁶¹. This elevation was found in imprinted chicks but not in light-reared or dark-reared controls⁶². Specific suppression of this elevation by RNA interference also impaired imprinting⁶³.

Training with an imprinting stimulus for 1 h leads to a learning-related up-regulation in the IMM of immunoreactivity for Fos, the protein product of the immediate-early gene *c-fos*⁴³. The extent of the increase in strongly imprinted chicks is similar irrespective of whether the chicks are trained for 1 h or 15 min⁶⁴. The learning-related increase in *c-fos* expression is thus likely to be triggered early in the imprinting process⁶⁵. A powerful tool for the study of the expression of this and other genes is the introduction of the relevant DNA into the IMM by electroporation⁶⁶. Using this technique coupled with bioluminescence imaging, Yamaguchi et al⁶⁷ confirmed that *c-fos* expression in the IMM increased with preference score and approach activity and that the expression of two further immediate-early genes (*zenk* and *Arc/Arg3.1*) was also up-regulated in imprinted chicks relative to dark-reared controls.

A pathway by which visual information might reach the IMM during imprinting has been described by Nakamori et al⁶⁸, who suggest that visual information from the thalamofugal pathway enters the IHA region of the visual wulst in the forebrain (the homologue of the mammalian primary visual cortex) and then passes to the hyperpallium dorsale before entering the IMM.

Electrophysiological studies

Chicks can be strongly imprinted to a rotating, internally illuminated red box or blue cylinder by exposure to either of these stimuli for two hours; see Bolhuis et al⁶⁹ for descriptions of the imprinting stimuli. Visual imprinting is particularly powerful if, during training, the visual stimulus is accompanied by a recording of the maternal call of a hen⁷⁰. It can be demonstrated that training has caused the chick to learn about visual features of the stimulus by testing the chick's preference in the absence of the maternal call. In untrained chicks, 10-20% of neurons in the IMM were found to show a significant change in firing rate in response to either visual stimulus alone (i.e. with no maternal call). In trained chicks with high preference scores, the percentage of neurons in the IMM specifically responsive to the visual imprinting stimulus rose to more than double the untrained value, in both the left IMM⁷¹ and the right IMM⁷². Some evidence was found for differences in the electrophysiological behaviour of the two sides of the IMM. For example, responsiveness to the novel stimulus was significantly reduced in the right IMM of the trained chicks but not in the left⁷³. Horn et al⁷⁴ studied the time course of neuronal responsiveness in the IMM during and after imprinting. After one or two hours' training there was a significant increase in number of neurons responsive to the imprinting stimulus (IS). This proportion then decreased before rising again to approximately three times the baseline value by the end of the experiment, approximately 24 h after the start of training. Individual neurons that had become responsive to the imprinting stimulus showed temporal fluctuation in their responsiveness to the IS. Many neurons, having acquired a strong responsiveness to the IS after one hour's training, lost it again after training for a second hour. The results prompted the authors to predict that the same neurons would regain their responsiveness to the IS by the end of the experiment the following day, approximately 24 h after

the start of training. This prediction was found to be correct by Jackson et al⁷⁵, measured the responsiveness of these neurons to the training and novel stimuli during and after training up to 19.5 h after the start of training. They found that the responsiveness of IMM neurons to the IS did indeed wax and wane. Moreover, as predicted by Horn et al⁷⁴, responsiveness to the IS was found to return almost completely by the end of the experiment. Jackson et al⁷⁵ also found that for IS-responsiveness to be retained it was necessary for chicks to be able to sleep without interruption during a six-hour period shortly after training (named Session 1 in Fig 2). If, during this period, chicks were prevented from sleeping continuously by slowly turning the running wheel through one revolution for one minute at random times once every 30 min, not only was responsiveness to the IS eventually lost but at the same time the chicks appeared amnesic for the imprinting stimulus (Figure 3). These effects were observed despite the fact that the chicks disturbed during Session 1 were rested during Session 2, a six-hour period between 12.5 and 18.5 h after the start of training (Figure 3). Provided that chicks were rested during Session 1, a high level of neuronal responsiveness to the imprinting stimulus returned, irrespective of whether chicks were disturbed during Session 2⁷⁵ or rested during Session 2⁷⁴. Taken together, the results indicate that a certain amount of undisturbed sleep during Session 1 is essential for both stabilization of specific neuronal responsiveness to the imprinting stimulus and for memory of the stimulus. Analysis of the electroencephalogram (EEG) recorded from the IMM showed a significant increase in the proportion of energy in the 4-6 Hz (low frequency theta) band 2, 3 and 4 h after the start of Session 1, restricted to the group of chicks that remained undisturbed during this period. It is not yet known whether this increase in low frequency theta activity occurred during sleep or while the animals were awake. These results provide an opportunity in further work to investigate the neural mechanisms whereby sleep enhances memory consolidation⁷⁶. Moreover, the results suggest that sleep-dependent memory consolidation involves a phase during which neuronal responsiveness, and therefore possibly synaptic efficacy, shows marked instability for several hours.

Figure 3 near here

As noted above, imprinting to a visual stimulus is particularly strong if this stimulus is accompanied by the maternal call of a hen during training. Approximately 24 h after the start of training, neuronal responsiveness in the IMM to the visual component of the stimulus alone is increased. An increase was also found in the proportion of neurons responsive both to the compound training stimulus (i.e. simultaneous presentation of the visual training stimulus and the maternal call) and to the visual stimulus when presented alone. In contrast, the proportion of IMM neurons responding to the compound stimulus but *not* the visual component alone decreased, evidently due to a decrease in responsiveness to the auditory component⁷⁷. Interestingly, neuronal responsiveness to a compound stimulus comprising the familiar visual component and a *novel* maternal call was found by Town and McCabe⁷⁸ to be increased. When chicks themselves (with their vocalisations) were used as imprinting stimuli, neuronal responsiveness in the IMM to the familiar chicks with vocalisations was reduced relative to responsiveness to unfamiliar chicks with vocalisations²³. Social rearing of chicks in groups, presumably leading to their becoming imprinted to each other, reduced their mean preference for an artificial stimulus (a red box or a blue cylinder) to which they had previously been imprinted. Neuronal responsiveness in the IMM to the familiar artificial stimulus was also reduced by social rearing⁷⁹. There are thus parallels between the social preferences formed through imprinting and neuronal activity in the IMM. The effects appear complex and there is evidence for disparity in the processing of information acquired via different modalities.

AUDITORY IMPRINTING

Domestic chicks will develop a preference for the maternal call of a hen and for rhythmic tone stimuli after exposure to either of these types of stimulus. Neural changes following imprinting to tone stimuli have been detected in the medio-rostral nidopallium/mesopallium (MNM; formerly the medio-rostral neostriatum/hyperstriatum ventrale or MNH), and include increased uptake of 2-fluorodeoxyglucose, reduction in numerical density of synapses, and changes in glutamate release and electrophysiological activity⁸⁰⁻⁸².⁸² Evidently memory processing after training involves different brain regions, depending on the modality employed for the imprinting procedure.

LATERALISATION

It has long been clear that there is a hemispheric asymmetry in the processing of information following imprinting training^{8,73}. Rogers et al⁸³ have demonstrated an asymmetry in visual pathways in the recently-hatched chick, which is dependent on asymmetrical visual stimulation of the two eyes during the last few days of incubation. However, the functional asymmetries demonstrated after imprinting and described above and in Figure 2 arise in the absence of such stimulation. Which is not to say that additional symmetries cannot be induced experimentally. When asymmetric visual stimulation is applied from day 19 of incubation (hatching occurs on day 21), functional reorganization of the nervous system occurs: illumination of either the left or right eye and occlusion of the opposite eye followed by visual imprinting causes the forebrain hemisphere ipsilateral to the illuminated eye to become critical for imprinting⁸⁴.

Hemispheric asymmetry of learning-induced changes in the brain of the chick may be very marked, as in the case of the effect of imprinting training on the numerical density of NMDA receptors in the IMM; in this case, no significant effect of training was observed in the right IMM whilst there was a strong learning-related effect on the left side and a significant interaction between side and training condition⁴¹. In other cases, the asymmetry is less marked⁴⁵ but if there is evidence for an asymmetry, the predominant effect of imprinting is on the left side of the IMM. For some measurements, no asymmetry has been found, for example in the expression of Fos-like immunoreactivity after imprinting training for one hour⁴³. Moreover, the right and left sides of the IMM both display prolonged increases in neuronal responsiveness to the imprinting stimulus after training. However, in so far as one particular measurement, be it Fos expression or electrophysiological responsiveness, displays only one aspect of the processes in the IMM, asymmetries may still exist: for example the left and right sides might contain the same number of affected cells, but the proportions of excitatory and inhibitory neurons on the two sides may differ, as may the ways in which neurons are interconnected.

Hemispheric asymmetry in the IMM is very important in the processing of information following imprinting. The mean length of the postsynaptic density of axospinous synapses in the left IMM increases after training, consistent with long-term storage of information about the imprinting stimulus in the left IMM. No such morphological change was observed in the right IMM⁴⁰.

Figure 4 near here

The finding of a change in synaptic morphology prompted a series of ablation experiments to determine the roles of the two sides of the IMM. The results indicated that the left IMM has a storage function whereas the right IMM, as well as being a store, is necessary for retention to be sustained by a supplementary storage area outside the IMM, termed S', over a period lasting several hours after the end of training⁷³. See Figure 4 for an explanation of these experiments.

Both the IMM and S' can sustain retention, but S' also has a mediational function. That is, it permits information acquired through imprinting to affect subsequent associative learning. If chicks are exposed to two imprinting stimuli sequentially in close temporal juxtaposition, say 15 s apart ("mixed training"), they subsequently learn to discriminate between the two stimuli more slowly than if the exposure to the two stimuli is respectively in two consecutive blocks separated by more than 30 min ("separate training")^{85, 86}.⁸⁵ By lesioning the IMM at different times after the end of training, Honey et al⁸⁶ prepared chicks that either had the left IMM intact and no S' (lesion right IMM ≤ 1 h after the end of training; cf Figure 5), or no IMM and S' intact (lesion IMM bilaterally 4-6 h after training; cf Figure 5). Chicks with mixed training learned the visual discrimination more slowly than separately trained chicks, only when storage in S' was allowed to occur. That is, only those chicks in which retention was sustained by S' behaved in the same way as intact chicks; if S' was not active, mixed training did not affect the rate of discrimination learning. It was suggested that chicks with mixed training and operational S' had classified the two stimuli together and that this was the reason for the interference with acquisition of the visual discrimination task⁸⁶. If S' is required for the classification together of two imprinting stimuli presented in close temporal sequence, it may be essential for the process whereby a chick, by being exposed to different views of a mother hen in close temporal juxtaposition, learns that these views are in fact different versions of the same object and thus warrant the same response, namely approach and filial behaviour⁸⁷.

Figure 5 near here

Further evidence of functional lateralization has arisen from reports of asymmetrical use of sensory pathways during and after imprinting, and from experimental restriction of sensory input to one side of the brain. These approaches are particularly informative in the study of avian behaviour because of birds' completely crossed optic chiasm: the output of each eye projects to the opposite side of the brain. By studying the use of the right and left eye in defined experimental situations, and by patching one eye to restrict visual input to the other, one may gain insight into the functions of the left and right 'eye systems', which include the visual pathways emanating from the right and left thalamus and optic tectum respectively. The eye predominantly used by chicks exposed to an imprinting stimulus changes as the chicks become familiar with the stimulus⁸⁸, suggesting changes in the mode of processing as imprinting progresses. Subsequent monocular occlusion indicated that the right and left eye systems perform different functions: chicks with the right eye patched, and thus using the left eye system, could discriminate between familiar and unfamiliar chicks. Chicks using the right eye system did not discriminate between familiar and novel objects unless the distinction between them was particularly marked⁸⁹. Recognition behaviour was found to be influenced by sex (male chicks preferred novel individuals and females preferred familiar individuals) and time: predominant use switches between the left- and right-eye systems at different times after learning, suggesting distributed storage of information between brain hemispheres and temporal

constraints on access by one hemisphere to the contents of the other during consolidation⁹⁰; see Rogers⁹¹ for a general review.

PREDISPOSITIONS

Although imprinting is characterised by learning, preferences for particular stimuli may occur in a young animal that has had no previous experience of these stimuli. Such preferences are called predispositions and can bias the animal's behaviour in such a way as to make imprinting more effective. For example, domestic chicks exposed to mild stress (e.g. handling) during the second day of life, were found to develop a preference for the head region of an adult fowl, duck or polecat⁸ relative to an artificial red stimulus that by itself elicits vigorous approach. This predisposition, unlike recognition memory arising from imprinting, is not abolished by bilateral ablation of the IMM⁹² and is intensified by androgen treatment⁹³. There is a sensitive period for the induction of the predisposition by mild stress and the predisposition is sensitive to degradation of adrenergic transmission by the drug DSP4^{94,95}. In the presence of mild stress, such as might be caused by isolation from the mother hen, the predisposition evidently directs the chick's attention to objects roughly resembling an adult conspecific, facilitating imprinting to that individual. This phenomenon may have adaptive value where the chick would otherwise continue in isolation, whereupon its chances of survival may be slim⁹⁶. A similar predisposition for crudely specified faces has been found in human neonates⁹⁶, and the chick appears to be a good animal model of the human system. Accordingly, the predisposition in the chick has been shown to be for a simple representation of a chick's face, a visual stimulus closely analogous to the stimulus configuration for which a predisposition occurs in human subjects⁹⁷⁻⁹⁹. A further predisposition has been described in chicks for dot patterns on a visual display exhibiting biological motion (e.g. resembling walking), over matched dot patterns that do not move in a biologically meaningful way¹⁰⁰. Most human babies also prefer such stimuli^{101,102}. When exposed to images of two objects, one evidently self-propelled by seeming to cause the motion of the other, chicks preferentially imprint to the 'self-propelled' object¹⁰³. Such results indicate that features characteristic of living, as opposed to inanimate, objects contribute significantly to the potency of an imprinting stimulus.

BOX 1

The memory underlying imprinting strikingly resembles recognition memory studied in human and non-human animals:-

- (i) Memory encoding requires no additional stimulus to act associatively as a reinforcer or unconditional stimulus^{2, 104}.
- (ii) The memory can be used flexibly, not only to reproduce behaviour acquired during training, but also to influence more versatile behaviour. For example, sequential exposure to two different imprinting stimuli in close temporal juxtaposition can modify the rate at which the chicks subsequently learn to discriminate between the same two stimuli, an effect interpreted as being due to the chicks classifying the stimuli together^{85, 86}. The suggestion is that new representations are

established after the rapidly juxtaposed exposure, which can subsequently be utilised in novel situations. Flexible use of recognition memory in normal human subjects may be demonstrated by the learning of paired associates, such as several pairs of unique playing cards; normal subjects can easily reproduce the pairings from a cluster of all the test cards presented simultaneously in random positions. In contrast, amnesic subjects, i.e. with severely impaired recognition memory, can learn the card pairings but are unable to use this information flexibly to reproduce the pairings from the random cluster¹⁰⁵.

(iii) Acquisition and retention of the memory is selectively abolished by lesions that have no effect on other types of learning such as simple conditioning^{38, 106, 107}.

It is not yet clear whether memory arising from imprinting can be fractionated into components corresponding to recollection and familiarity, this having been proposed as a characteristic of recognition memory¹⁰⁸.

Conclusion

Imprinting has been studied extensively, from the perspectives of ethology, experimental psychology, behavioural ecology and neuroscience. It continues to contribute to all of these disciplines and has proved particularly productive in the study of the neural mechanisms of learning and memory. A complete understanding of imprinting will require information from many lines of enquiry: it seems likely that behavioural observations, evolutionary considerations, learning theory and the analysis of neural mechanisms will be required, including the many complementary experimental approaches that have become available in each field. A major challenge is that of finding the appropriate level of analysis in each contributing discipline. An ethogram needs to be sufficiently detailed to be useful and yet beyond a certain level, extra detail can be unhelpful. Microarray technology can investigate gene expression systematically across the genome, yet combine the statistical problems of bioinformatics with those of the observer of behaviour and hypotheses amenable to decisive testing can prove elusive. There are of course ways of simplifying the problems: experience suggests which behavioural measurements are likely to yield the most useful information, and which genes are likely to yield the best insights into synaptic function. Anatomical localisation of neural systems within the forebrain facilitates interpretation of the effects of localised drug injection and normal brain function is remarkably resilient to the introduction of microelectrode probes. Multidisciplinary analysis of imprinting therefore has a promising future.

Benefits of the multi-disciplinary study of imprinting have arisen at several different levels of experimental analysis, some of them unexpected: the lesion experiments designed to investigate hemispheric asymmetry in the IMM^{37, 38, 109, 110} were prompted by the demonstration of hemispheric asymmetry in synaptic morphology following imprinting^{39, 40}. These lesion experiments led to the discovery of a predisposition to prefer faces⁹⁶⁻⁹⁹ and demonstrated the existence of a brain region S¹, which is necessary for the flexible use of information acquired through imprinting⁸⁶. Neurobiological analyses thus illuminated issues that had arisen in the ethological literature and behavioural results reciprocally raised problems that required a mechanistic explanation. Such experiments exemplify the advantages of giving serendipity a chance.

It is possible that an understanding of the neural mechanisms underlying imprinting and related phenomena, such as behavioural sensitive periods, predispositions and the role of sleep in memory consolidation will facilitate understanding of these processes at a fundamental level, across a range of species. A number of important questions arise: why do the structure and connectivity of the avian and mammalian forebrains³⁶ differ so markedly, and yet evidently have similar functions? What are the definitive properties of recognition memory? How does sleep act to consolidate memory? What controls the duration of a sensitive period? It is possible that imprinting, occurring as it does in both birds and mammals, will contribute to the solutions of these problems and possibly help show for which functions the avian and mammalian systems of neuroanatomical and functional organisation are optimized. It would be surprising if the ways in which neuronal networks subserve memory differ greatly between species. Finally, the practical advantages of imprinting in the chick for the analysis of learning and memory (developing [and thus plastic] central nervous system; rich behavioural repertoire; ease of obtaining large sample sizes; no feeding necessary for several days post-hatch) make it a particularly attractive system for elucidating mechanisms of behaviour and the modification of behaviour by experience.

Notes

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Figure captions

Figure 1

Plot illustrating learning-relatedness of a physiological measurement in the brain after imprinting. Data are taken from ref ⁴³, in which the number of Fos-positive nuclei (square root- transformed to normalize the data) are plotted against preference score, a measure of preference for the imprinting stimulus and thus of the strength of imprinting/learning. Nuclei were counted in a standard sampling

frame placed over the IMM region in a histological section. Each point represents data from one chick. The least squares regression line has been fitted. The lower horizontal dashed line estimates the value of the ordinate corresponding to the 'no preference' score of 50 (characteristic of chicks showing no learning). This estimate was not significantly different from the mean value for untrained chicks, which is represented by the open circle (the error bars represent ± 1 SEM, $n = 16$). The upper horizontal dashed line gives the estimated value of the ordinate corresponding to the maximum preference score attained in the experiment (characteristic of strongly imprinted chicks). This estimate was significantly greater than the mean value for untrained chicks. The estimates shown by the horizontal dashed lines are based on interpolation of the regression line. The thick bars on the Y axis depict \pm one standard error of each estimated value.

Figure 2

Summary of learning-related biochemical changes in the IMM and the times after the end of imprinting training at which they were detected. In some cases the changes were lateralised and when this was the case, the learning-related effect was always stronger in the left IMM. An effect is reported as lateralised if either (i) there was a significant interaction between the side of the IMM and strength of learning (measured either by regression of the biochemical change on preference score, or by a difference between good and poor learners); or (ii) there was a significant effect of training on one side of the IMM but not on the other.

Figure 3

a, mean proportions of neurons \pm SEM in the IMM of domestic chicks that were responsive to a visual imprinting stimulus (IS - a red box), plotted against time since the start of the experiment⁷⁵. Chicks were exposed to the IS for two one-hour periods, denoted by Train1 and Train2. Neuronal responsiveness to the IS was tested by neuronal tests NT1 – NT4. Chicks remained in running wheels throughout the experiment. Filled squares represent data from chicks (Rest-First group) that were allowed to rest in darkness during the six-hour period labelled 'Session 1'. Open circles represent data from chicks (Disturbed-First group) that were prevented from sleeping continuously during Session 1 by a single revolution of the running wheel (duration one minute) delivered at random every 30 min during Session 1. During Session 2, which was also six hours in duration, the Rest-First chicks were disturbed as described above and the Disturbed-First chicks were allowed to rest. At test NT4, neuronal responsiveness to the IS in the Rest-First group had risen significantly to a maximum value that was significantly higher than neuronal responsiveness to the IS in the Disturbed-First group; in this latter group, the responsiveness had collapsed at NT4. The high level of responsiveness in the Rest-First group was due to the fact that this group was rested during Session 1 rather than disturbed during Session 2, since a high level of responsiveness at NT4 was also found if chicks were rested during Session 2⁷⁴.

b, neuronal responsiveness to a novel stimulus (a blue cylinder) in the Rest-First and Disturbed-First groups that contributed data to panel A. In both groups, only approximately 10% of neurons in the IMM responded to the imprinting stimulus during all of the neuronal tests NT1 – NT4.

c, mean preference scores \pm SEM for the Rest-First and Disturbed-First chicks measured at the end of the experiment. The Rest-First chicks had a preference score significantly ($P = 0.03$) greater than 50, indicating that they had become imprinted. In contrast, the Disturbed-First chicks had a mean preference score that was not significantly different from the 'no preference' level of 50.

Disturbance during Session 1, as well as causing a drastic reduction of neuronal responsiveness to the imprinting stimulus at NT4 (panel A, filled squares), also reduced the mean preference score to a level at which no evidence of memory for the imprinting stimulus remained.

Modified from reference ⁷⁵.

Figure 4

Summary of the results of experiments^{38, 109} in which the IMM was ablated either unilaterally or bilaterally after imprinting training. Lesions in the IMM are represented as oval shaded areas and the left and right forebrain hemispheres are denoted by "L" and "R" respectively. "Result 1" gives the result of a preference test following IMM ablation under general anaesthesia ≤ 3 h after training. "Result 2" is derived from a preference test after ablation under general anaesthesia ~ 24 h after training. a, bilateral ablation of the IMM ≤ 3 h after training results in amnesia for the imprinting stimulus. b, ablation of first the right IMM and then the left IMM gives amnesia after the second lesion. c, in contrast, ablation of the left and right IMM in the reverse order results in *retention* of the preference acquired through imprinting. d, if both the left and the right IMM are intact for ~ 24 h after training, the preference acquired through imprinting is retained after subsequent ablation. In summary, if the right IMM is intact for a sufficient time after training, storage of information about the imprinting stimulus occurs outside the IMM, in a region termed S'. If the right IMM is lesioned ≤ 3 h after training, retention is dependent on the remaining left IMM and there is no evidence of S' being functional.

Figure 5

Experiment demonstrating the mediational function of region S' in the chick brain. On Day 1, all chicks received a total of 100 presentations of imprinting stimulus A and 100 presentations of imprinting stimulus B during two 53-min sessions of imprinting training. In the Mixed training condition, both sessions contained 50 presentations of each of the stimuli, A and B, in a quasi-random order. For chicks in the Separate training condition, one session contained 100 presentations of A and the remaining session contained 100 presentations of B. For half of these chicks, A was presented in Session 1 and B in Session 2; for the remaining chicks, B was presented in Session 1 and A in Session 2. Chicks then received lesions, either unilaterally in the right IMM < 1 h after training (Group R-IMM, preventing storage in S') or bilaterally in the IMM 4 – 6 h after training (Group B-IMM, allowing storage in S'). On the next day, all chicks received visual discrimination training in which they were rewarded for approaching stimulus A. Chicks in Group B-IMM receiving Mixed training (framed) learned the discrimination significantly slower than the other three groups, whose acquisition rates did not differ significantly from each other. Thus, learning during the Mixed training session impaired subsequent discrimination learning if S' remained intact. This impairment was interpreted as S' mediating the classification together of A and B following mixed training and thereby interfering with the acquisition of a discrimination between A and B. From Honey et al⁸⁶.

Tables

[Please insert any tables here]

Further Reading/Resources

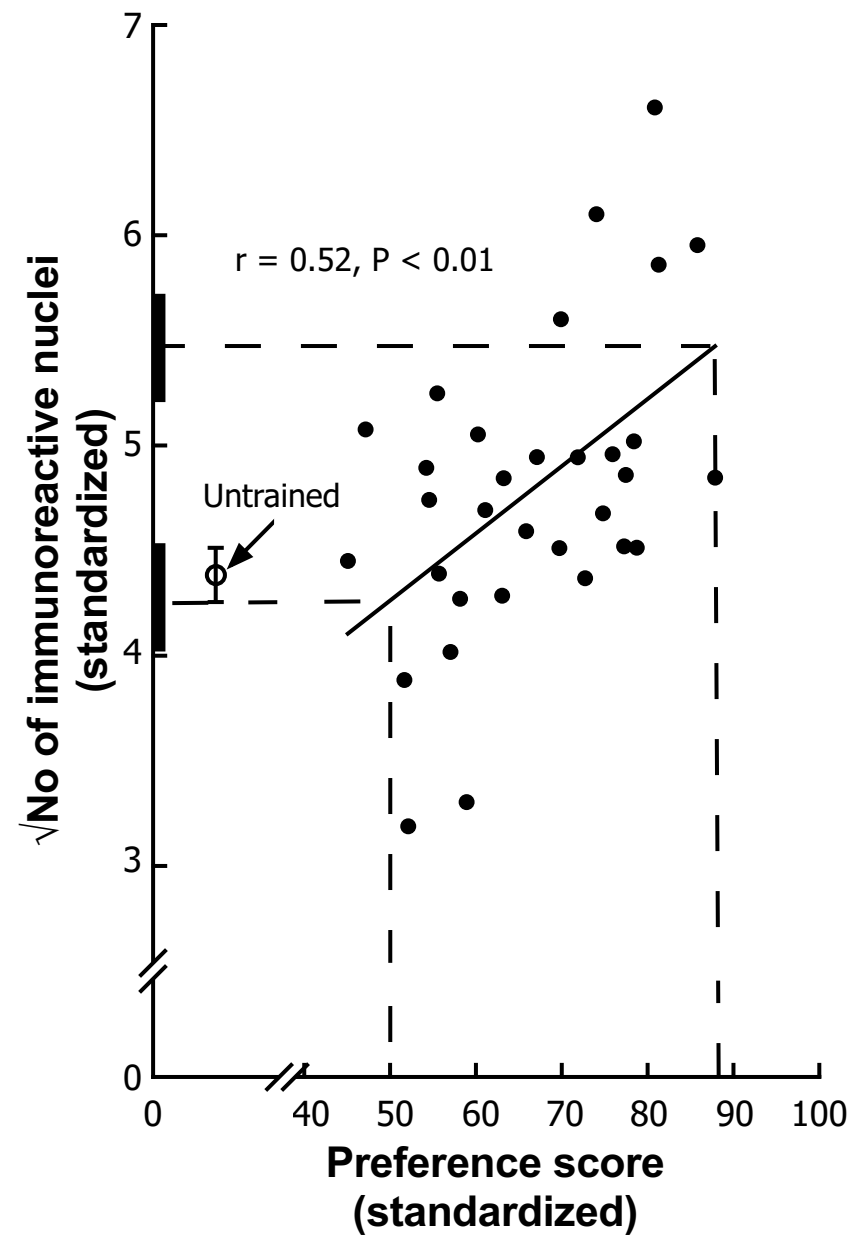
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Related Articles



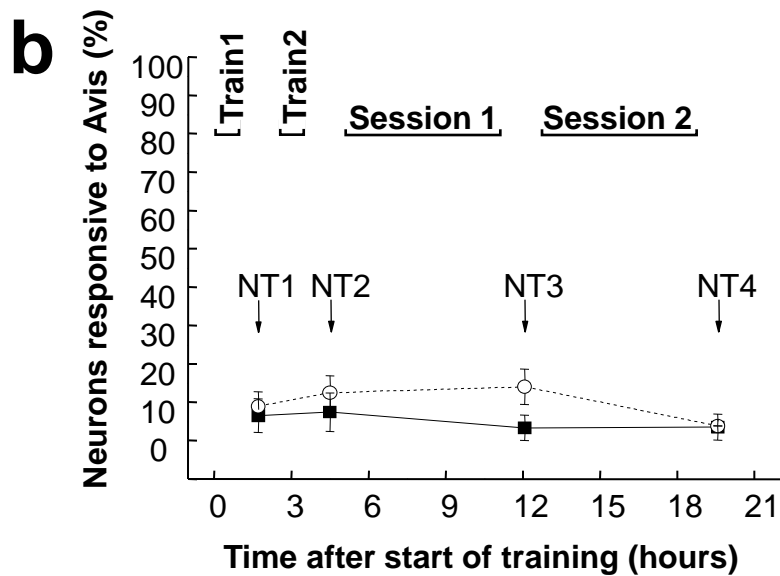
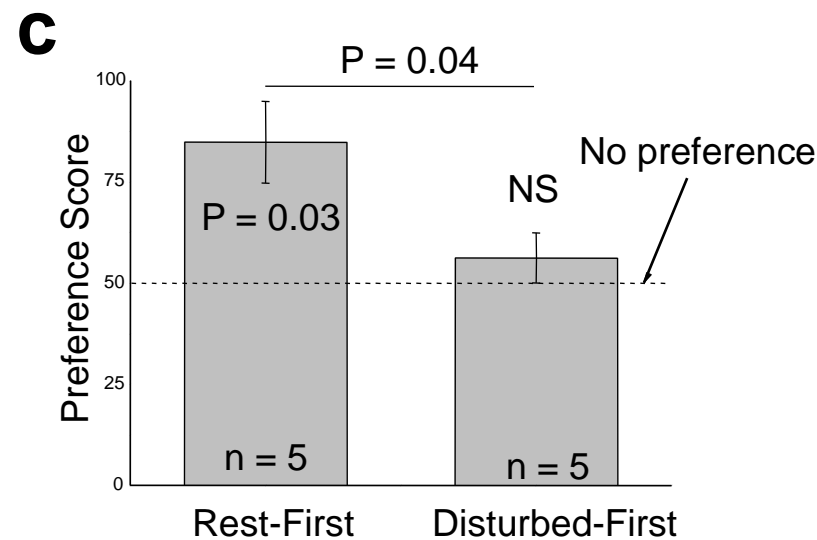
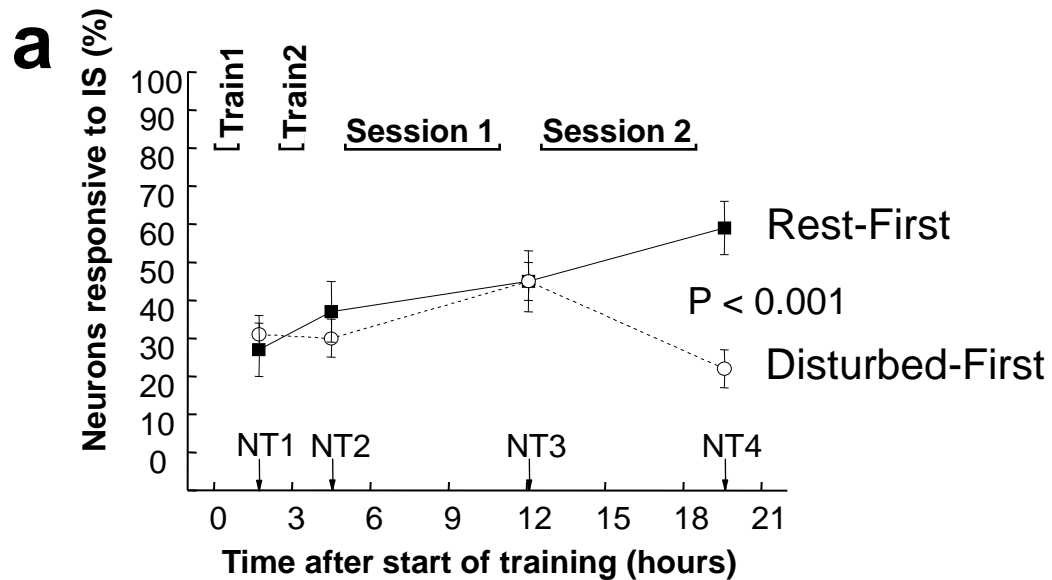
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Article ID	Article title
e.g., COGSCI-235	Imprinting



Time after end of training (h)

- 1 *Changes in: neuronal activity; gene expression; kinase activation and activity.*
Increased Fos expression in neurons immunopositive for GABA, taurine and parvalbumin but not calbindin (i.e. a sub-set of inhibitory neurons)^{43, 64, 65, 111}
Increased CaMKII autophosphorylation⁴⁵
Increased MARCKS phosphorylation (lateralised)⁴⁸
Increased expression of MARCKS mRNA⁴⁹
Increased phosphorylation of AMPA receptors (lateralized)¹¹⁷
- 1 and 3.5 *Change in: releasable neurotransmitter pool.*
Increased calcium-dependent, potassium-stimulated release of GABA and taurine (lateralised)^{112, 113}
- 3-3.5 *Change in: morphology of putative excitatory synapses.*
Increased size of postsynaptic density of axospinous neurons (lateralised)^{39, 40}
- 7-8 *Change in: glutamate receptor number.*
Increased NMDA receptor number (lateralised)^{41, 114}
- 10 *Change in: releasable neurotransmitter pool; receptor subunit mRNA.*
Increased calcium-dependent, potassium-stimulated release of GABA and taurine (lateralised)^{112, 113}
Down-regulation of GABAA receptor $\gamma 4$ subunit mRNA¹¹⁵
- 22-25 *Changes in: synaptic morphology; vesicle endocytosis; neural cell adhesion; cell-cell signalling; cytoskeleton; oxidative phosphorylation.*
Increased size of postsynaptic density of axospinous synapses (lateralised)⁴⁰
Increased amount of:
Clathrin heavy chain protein (lateralised)⁵¹
Neural cell adhesion molecules (lateralised)¹¹⁶
Non-phosphorylated (membrane-bound) MARCKS (lateralised)^{50, 53}
Amyloid precursor protein (lateralised)⁵⁰
Cytochrome c oxidase subunits I and II (lateralised)⁵⁹



**Lesions \leq 3 h
after training**

Result 1

**Lesions \sim 24 h
after training**

Result 2

a

Train



Amnesia

Amnesia

b

Train



Retention



Amnesia

c

Train



Retention



Retention

d

Train

Retention



Retention

Imprinting training		Lesion
Session 1	Session 2	
0	0	Control
0	1	Medial prefrontal cortex
0	2	Hypothalamus
0	3	Ventral tegmental area
0	4	Nucleus accumbens
0	5	Amygdala
0	6	Hippocampus
0	7	Caudate putamen
0	8	Pituitary gland
0	9	Adipose tissue
0	10	Skeletal muscle
0	11	Blood
0	12	Uterine horn
0	13	Ovary
0	14	Vagina
0	15	Rectum
0	16	Anus
0	17	Penis
0	18	Scrotum
0	19	Testis
0	20	Epididymis
0	21	Vas deferens
0	22	Utricle
0	23	Seminal vesicle
0	24	Prostate gland
0	25	Bladder
0	26	Urethra
0	27	Penile urethra
0	28	Glans penis
0	29	Prepuce
0	30	Perineum
0	31	Anal canal
0	32	Rectoanal junction
0	33	Sigmoid colon
0	34	Cecum
0	35	Ileocecal junction
0	36	Ileum
0	37	Caecum
0	38	Ascending colon
0	39	Transverse colon
0	40	Descending colon
0	41	Sigmoid colon
0	42	Rectum
0	43	Anus
0	44	Penis
0	45	Scrotum
0	46	Testis
0	47	Epididymis
0	48	Vas deferens
0	49	Utricle
0	50	Seminal vesicle
0	51	Prostate gland
0	52	Bladder
0	53	Urethra
0	54	Penile urethra
0	55	Glans penis
0	56	Prepuce
0	57	Perineum
0	58	Anal canal
0	59	Rectoanal junction
0	60	Sigmoid colon
0	61	Cecum
0	62	Ileocecal junction
0	63	Ileum
0	64	Caecum
0	65	Ascending colon
0	66	Transverse colon
0	67	Descending colon
0	68	Sigmoid colon
0	69	Rectum
0	70	Anus
0	71	Penis
0	72	Scrotum
0	73	Testis
0	74	Epididymis
0	75	Vas deferens
0	76	Utricle
0	77	Seminal vesicle
0	78	Prostate gland
0	79	Bladder
0	80	Urethra
0	81	Penile urethra
0	82	Glans penis
0	83	Prepuce
0	84	Perineum
0	85	Anal canal
0	86	Rectoanal junction
0	87	Sigmoid colon
0	88	Cecum
0	89	Ileocecal junction
0	90	Ileum
0	91	Caecum
0	92	Ascending colon
0	93	Transverse colon
0	94	Descending colon
0	95	Sigmoid colon
0	96	Rectum
0	97	Anus
0	98	Penis
0	99	Scrotum

Discrimination training, A vs B

Mixed training A,B,B,A... B,A,A,B...

Reward approach to A only

Separate training A,A,A,A... B,B,B,B...
or B,B,B,B... A,A,A,A...

Mixed training	A,B,B,A...	B,A,A,B...
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Reward approach to A only

Separate training A,A,A,A... B,B,B,B...
or B,B,B,B... A,A,A,A...